Supplementary Note 1

Clonal assignments were made based on matching V and J regions, identical HCDR3 length, and ≥ 85% sequence similarity throughout the HCDR3 sequence. This identity threshold was chosen after a thorough analysis of samples was conducted to determine an optimal metric for ASC clonal identification that would identify clonally related sequences with different degrees of mutation while excluding similar sequences of distinct clonal origin. To that end, CD138– ASCs from an influenza-vaccinated sample, 7 days post-vaccination, was sorted into 3 separate tubes of 5 × 104 cells each. The sequences obtained from these 3 tubes were then compared to similar numbers of CD138+ ASCs sorted from the same subject and CD138– ASCs sorted from a separate subject. Using the split samples from the same subject as positive markers and the\ sample from the separate subject as a negative marker, we analyzed sequences both through our automated analysis platform and also manually to determine which percentage of HCDR3 identity proved most efficient. A separate metric was also used which involved, in addition to the previous criteria, the requirement of identical junctional segments, identified as the last 3 nucleotides in the V region, first 3 nucleotides in the HCRD3, last 3 nucleotides in the HCDR3 and first 3 nucleotides in the J region. Results are presented in Supplementary Figs. 2, 3. It was determined that using greater than or equal to 85% HCDR3 similarity to identify clones resulted in a large overlap between identical samples (for same subject splits samples: 47.0% lineage connectivity between Tube 1 and 2, 46.1% between 1 and 3, and 46.9% between 2 and 3) and minimized the connectivity between different subjects (average 0.3% connectivity). In these examples, any difference between separate tubes from the same subject is likely due to limited sampling depth as 100% connectivity was found between the separate tubes of the same individual when only the top 50% of clones was considered. Any role for PCR sequencing error was examined by testing separate sequencing runs of the same identical samples. We found that the vast majority of errors concentrated on the smallest clones without significant difference in the overall clonality or the identity of the largest clones.

In all, we considered that, given the predictable accumulation of somatic hypermutation that occurs as naïve and memory cells differentiate into ASCs, the use of complete or near complete sequence identity does inevitably diminish the degree of actual clonal connectivity between any two given populations. The 85% threshold value finally chosen takes this factor into account by allowing the HCDR3 within a single clone to accumulate a level of somatic hypermutation commensurate with the average mutation rate found in the CDR1 and CDR2 of all ASC samples. In contrast, identity thresholds higher than 85% frequently split into multiple smaller clones, clonally related sequences verified by sequence alignments demonstrating shared and unique mutations throughout the entire V(D)J rearrangement. This limitation was clearly illustrated in analysis of antigen-driven oligoclonal responses triggered by vaccination (Supplementary Fig. 2). The same problem was identified when a requirement for identical junctional segments was imposed.